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(54) Title: PIEZOELECTRIC DEVICE FOR DETECTION OF POLYNUCLEOTIDE HYBRIDIZATION

(57) Abstract

A system for detecting polynucleotide hybridization. A polynucleotide is immobilized on a surface of a piezoelectric crystal. The resonance frequency of the piezoelectric crystal is measured through means for determining the resonant frequency of a piezoelectric crystal. A separate source of polynucleotide is exposed to the polynucleotide-coated piezoelectric crystal for a sufficient length of time and under conditions suitable for hybridization. The resonance frequency of the crystal is then again measured, and the difference between the resonance frequency before and after the incubation period will indicate the extent of hybridization.

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PIEZOELECTRIC DEVICE FOR DETECTION
OF POLYNUCLEOTIDE HYBRIDIZATION

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The present invention generally concerns a system for
detecting polynucleotide hybridization. More particular-
ly, the invention pertains to a system for detecting
20 polynucleotide hybridization utilizing piezoelectric
crystals. The invention has particular application to
medical diagnosis, bacteriology, virology, pathology, and
biochemical and molecular biological research. For
example, the system of the present invention is useful for
25 detecting polynucleotide hybridizations such as DNA-RNA,
DNA-DNA and RNA-RNA hybridizations.

DNA and RNA are strands of nucleic acids character-
ized by a phosphodiester link between the 3' position of
30 one nucleic acid residue with the 5' position of the next
nucleic acid residue. Nucleic acids contain one or more
of the following bases: guanine (G), cytosine (C), adenine
(A), and either thymine (T) (occurring in DNA) or uracil
(U) (occurring in RNA).

35

In double-stranded DNA it is known that the pairing
of the DNA strands are complementary, i.e., the guanine of
one strand always pairs with the cytosine of the other
strand (G-C), and the adenine of one strand always pairs
5 with the thymine of the other strand (A-T). The same is
true for double stranded RNA with the exception that
uracil is substituted for thymine (A-U).

Pairing of single strands of polynucleotides occurs
10 through hydrogen bonds formed between complementary bases
of the nucleic acids. When heated sufficiently, the
paired polynucleotides will melt; i.e., the hydrogen bonds
are disrupted and the double strands are separated into
individual strands (denatured). The polynucleotide
15 strands tend to re-form duplexes once separated into
single strands through the re-forming of hydrogen bonds
between complementary bases.

The ability of DNA or RNA strands to reanneal with
20 complementary strands of polynucleotides has led to the
ability to determine base sequence homology through
hybridization. The extent of hybridization between a DNA
strand with a known base sequence, for example, and a
strand of polynucleotide from a different source, can
25 serve as a measure of species relatedness, and is useful
for the purpose, among others, of identifying bacteria and
viruses.

Several methods of detecting polynucleotide hybridi-
30 zation have been developed. One such method of detecting
polynucleotide hybridization relies on radiolabeling.
Other known methods make use of renaturation rates,
electron microscopic determination, and the use of fluoro-
chrome labeled polynucleotides in conjunction with fluo-
35 rescence microscopy. IgG specific for RNA:DNA hybrids has
also been used. Still other methods use nucleotide

derivatives which contain biotin, aminobiotin, lipoic acid
and other determinants attached covalently to the pyrimi-
dine or purine ring.

5   The techniques employed for detecting DNA or RNA
relatedness have been applied in research laboratories for
several purposes, such as identifying biochemically
atypical strains, classifying poorly studied groups of
organisms, justifying or invalidating proposed taxonomic
10   changes, determining the relationships between newly
described organisms and existing taxons, and for identify-
ing mixed cultures. Unfortunately, the application of
these techniques in clinical studies have for most pur-
poses been impractical. For example, in clinical studies
15   where DNA hybridization detection employs radiolabeling,
the technique has been shown to be impractical since it
lacks simplicity and rapidity, is expensive, and poses
potential safety hazards.

20   Generally, the present invention comprises a novel
component and method for the detection of polynucleotide
hybridization. A polynucleotide is immobilized on a
surface of a piezoelectric crystal and the resonance
frequency of the crystal is then noted. A variety of
25   means for determining the resonance frequency of a piezo-
electric crystal can be employed. Polynucleotide hybridi-
zation can then be detected by measuring the difference
between the resonance frequency of the piezoelectric
crystal before and after the prepared crystal is exposed
30   to a separate source of polynucleotide under suitable
hybridization conditions.

Piezoelectric Detection of Mass

Piezoelectricity is the electricity, or electric
polarity, resulting from the application of mechanical
5   pressure on a dielectric crystal. The concept of piezo-
electricity is well known. For example, peizoelectric
crystals are used in controlling frequencies in the
communications field, as selective filters in electronic
networks and also as sensors to accurately measure
10   temperature.

Certain crystals exhibit a piezoelectric effect,
i.e., compression of the crystals generate an electro-
static voltage across the crystals. Examples of these
15   types of crystals include quartz, ethylene diamine
tartrate, barium titanate, zincblende and the like. The
frequency of a crystal depends on its effective mass. The
resonant frequency of the crystal can be obtained with a
frequency meter attached to the output of an oscillator
20   circuit. If the mass of the crystal is altered, as for
example, by adding material to the surface of the crystal,
the resonant frequency of the crystal will change and can
be measured on a frequency meter. The frequency of the
crystal will lower proportionally to the amount of mass
25   added to the crystal.

Piezoelectric crystals are commonly produced from AT
or BT cut quartz plates (AT and BT refer to the orienta-
tion of the plate with respect to the crystal structure).
30   AT and BT cut crystals vibrate in a shear mode about an
axis parallel to the major surface, and have low or zero
temperature coefficients at the temperature of use. The
AT cut is superior to the BT cut in temperature coef-
ficient and mass sensitivity. A typical 9 MHz crystal is

a 10-16 mm disc, square or rectangle about 0.15 mm thick.
A 9 MHz, AT cut quartz crystal has proven very effective
in the practice of this invention.

5   Means for detecting the resonance frequency of a
crystal can be provided in a variety of ways. Generally,
a crystal will be interposed between electrode material,
the leads of which are connected to an oscillator circuit.
A frequency meter or the like attached to the output of
10   the oscillator circuit is used to measure the resonance of
the crystal.

      The electrode material need not be in physical
contact with the crystal. However, an efficient way of
15   preparing a piezoelectric crystal for use in the present
invention is to deposit electrode material on opposite
surfaces of the crystal. For example, on a typical quartz
crystal, the electrode material can be deposited on the
central region of opposing crystal surfaces. In addition,
20   a band of electrode material can be deposited on the
crystal surface to form a pathway from the electrode
material deposited on the central region of crystal
surface to the edge of the crystal where leads for con-
necting to an oscillator circuit can be attached.
25

      Any suitable electrode material can be used in the
practice of this invention. Such materials include, but
are not limited to, gold, silver, aluminum, nickel,
chromium, titanium tantalum and the like.
30

      The instrumentation employed, such as a simple
oscillator circuit, a frequency counter and a vacuum oven,
and the use of micro-electric circuitry should be
economically advantageous since it is inexpensive yet
35   effective. In addition, the present invention should
require only limited training to operate, especially since

a signal from a piezoelectric crystal is at a frequency
which can easily be displayed on a digital meter or
converted to a digital signal for communication to a
computer.

5

Bonding of Polynucleotides
to Piezoelectric Crystals

It is well known that polynucleotides will hybridize.
10 For example, denatured DNA plus its complement will hy-
bridize under the proper conditions and reassociate into
double-stranded DNA. Hybridization of a DNA strand occurs
only if incubated with its complement. Partial hybridiza-
tion can occur between single strands of related species.
15 The extent of hybridization corresponds to the extent of
species relatedness. For example, hybridization of
approximately 60% is considered to indicate a close
relation between species.

20   Polynucleotides can be chemically bonded directly to
a piezoelectric crystal surface or indirectly via a
material previously deposited on the crystal surface. The
crystal surface, for the purposes of this description,
includes the crystal surface per se or any material or
25 materials deposited on the surface of the crystal. Such
other material can be electrode material or a thin layer
of polymeric or other bonding material, such as intermed-
iate molecule links. For example, a synthetic polynucleo-
tide can be bonded chemically to the crystal surface to
30 which a second, naturally occurring, polynucleotide can be
attached by enzymatic ligation. More particularly, a
polynucleotide can be ligated by enzymatic reaction (DNA
or RNA ligase) to a different polynucleotide which is
covalently bonded by chemical reaction to a functional
35 group which is in turn chemically bonded to a substrate,
for example, a polymeric substrate.

Particularly effective polymeric substrates are those
polymers characterized by hydrolytically stable,
hydrophobic backbones substituted with reactive pendant
groups. For example, such polymers include, but are not
5   limited to, copolymers of ethylene or propylene and N-(6-
x-hexyl)-acrylamide, copolymers of styrene and p-x-methyl
styrene, copolymers of alkylated siloxanes and 6-x-hexyl
and alkyl substituted siloxanes, and similar polymers
where x is a reactive group such as amino, sulfhydryl,
10 iodo bromo, chloro, carboxyl, hydroxy, chloro carbonyl,
dimethylsilyl and similar groups which are capable of
combining with polynucleotides or derivatized
polynucleotides. Other polymers useful in the present
invention include, but are not limited to, poly(butyl
15 methacrylate), polyurethane and the like.

One of the most sensitive areas for immobilizing the
polynucleotides is the central portion of the crystal
surface.

20

In one embodiment of the present invention a polymer
solution (usually 5 to 15 ul) is pipetted onto the crystal
and the solvent is evaporated _in vacuo_. Depending upon
the polymer solution used and prior experience, the
25 crystal may be washed at this stage. The wash may consist
of a combination of solvent or solvents and solutions. A
typical wash would be with 100 ml of distilled water. As
described above, it may be desirable to attach the poly-
nucleotides to that portion of the crystal surface com-
30 prising the electrode material. In this instance, the
polymer can be applied substantially to that area of the
crystal surface.

The resonance frequency of the dry crystal can be
35 optionally measured at this stage. If this is done,
washing followed by drying _in vacuo_ and measurement of the

resonance frequency should be repeated until the resonance
changes by less than +/- 50 Hz between successive wash-dry
cycles. Prior experience may indicate the amount of
washing required to achieve a stable frequency (+/- 50 Hz)
5 at this stage. Indeed, prior experience may indicate that
no wash-dry cycle is required at this point. Whether a
wash cycle is needed will largely be determined by the
polymer and attachment method used. The reverse side of
the crystal may optionally be treated in the same way.
10

        A buffer solution (usually phosphate buffer, pH = 6
to 8) containing the polynucleotide to be immobilized is
pipetted onto the crystal surface. This solution may
contain additional linking reagents which react with
15 either the polynucleotide, or with the polymer, or with
both, to form chemical bonds between the polynucleotide
and the polymer or other surface material. In some cases
these reagents that can react with the polynucleotide, or
with polymer, or with both may be applied to the crystal
20 and dried before polynucleotide addition. Alternatively,
electromagnetic radiation may be used to activate pre-
existing functional groups on the polymer or the poly-
nucleotide, or both, to cause formation of chemical bonds
between the polynucleotide and the substrate.
25

        After reaction with DNA or RNA, the crystal is
repeatedly washed with distilled water and dried _in vacuo_
until the resonance frequency is stable within +/- 50 Hz.
The temperature of the wash may be elevated, but not so
30 high as to cause hydrolysis or other disruption of desir-
able bonds.

        After the desired polynucleotide has been coated on
the crystal surface, the crystal frequency can be re-
35 corded. This is done first by washing the crystal of all
soluble and nonadhering material, and then drying the

crystal in a vacuum under low heat. Afterwards, the
resonance frequency of the crystal is measured and re-
corded.

5   The piezoelectric crystals, nucleic acids and other
polymers used are relatively stable and inexpensive. It
is contemplated that prepared piezoelectric crystals can
be manufactured so inexpensively that one-time use will be
feasible. Thus, one distinct advantage of the present
10   invention is the ability to provide a variety of kits for
use in the detection of polynucleotide hybridization. For
example, such kits can include, as separate components, a
test crystal, a control crystal, polymer, polynucleotide,
linking reagent, electronic equipment, or any combination
15   of these. In addition, the kit can include the crystal
prepared to any extent desired, for example, with the
polymer or other substrate or linking material only, or
with the polynucleotide attached.

20

Piezoelectric Detection
of Hybridization

      An aqueous buffer solution containing a polynucleo-
25   tide is deposited on the surface of the crystal prepared
generally as described above. The polynucleotide in the
solution can be either single- or double-stranded. The
solution and crystal can be heated to a temperature
sufficient to melt any double-stranded polynucleotides to
30   single strands. Next, the temperature is brought to a
predetermined value optimum for the hybridization of the
reference polynucleotide. This incubation period is
allowed to continue for a predetermined amount of time
based on the polynucleotide involved. This period can
35   last from a few minutes to several hours depending on the
complexity of the polynucleotide.

In one embodiment, hybridization is carried out by
incubating on the surface of a crystal a buffered solution
of polynucleotide. Typically the solution will contain
about 1 milligram/milliliter of the polynucleotide to be
5   tested, but can have a substantially lower concentration.
A typical incubation temperature would be approximately
30° C below the melting temperature of the duplex to be
formed. The incubation time will vary from a few minutes
to several hours depending upon the complexity and molecu-
10   lar weight of the polynucleotide to be duplexed. A useful
temperature regimen involves heating the polynucleotide
solution above its melting point and applying the hot
solution to the crystal. Then the temperature is lowered
to the incubation temperature and held there for the
15   required time. Alternatively, a melted solution of DNA or
RNA can be rapidly cooled, i.e., quick cooled, to slow the
spontaneous association of complementary DNA or RNA
strands.

20   The quick cooled solution is then applied to the
crystal and incubated at the optimum temperature for
duplex formation. In many cases it is possible to use
solvent systems which lower the association temperature.
For example, use of 50% formamide allows effective incuba-
25   tion at lower temperatures in cases where an elevated
temperature would be required without formamide.

Typically 1 to 50 microliters of polynucleotide
solution is applied to one or both sides of the crystal.
30   The solution should be retained on the crystal during
incubation without significant evaporation. This can be
done, for example, by forming a silicone rubber reaction
chamber (dome) over the crystal's central region. The
reaction chamber can be made from a silicone gas chromato-
35   graph septum with the center portion removed and covered

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with a second intact septum. Alternatively, a reaction
chamber specially manufactured to accommodate piezoelec-
tric crystals can be used.

5   Following the incubation period, the crystal is
washed free of soluble and nonadhering material and dried
under a vacuum. If the applied solution contained a
polynucleotide which is the complement of the reference
polynucleotide, then the mass of polynucleotide on the
10 crystal surface should be increased. Generally, the
reference polynucleotide will be immobilized on the
crystal surface prior to incubation with the test
polynucleotide. However, it is possible to reverse this
order, i.e., immobilize the test polynucleotide on the
15 crystal surface and then incubate the crystal with the
reference polynucleotide.

     This system is capable of providing both qualitative
and quantitative detection of hybridization. The amount
20 of the increase in mass caused by hybridization of the
polynucleotides is generally measured with a frequency
meter. The extent of hybridization is evaluated from the
difference in resonance frequency before and after the
incubation step. For example, based on a 9 MHz crystal,
25 an approximately 0.8 hertz shift corresponds to a one
nanogram change in mass. The sensitivity of the detection
is a function of the fundamental frequency of the crystal.
A higher fundamental frequency of the crystal corresponds
to an increase in sensitivity, while a lower fundamental
30 frequency will give the opposite result.

     In addition, it is contemplated that this system will
provide numerous advantages over other known techniques,
especially in clinical work where rapid and accurate, yet
35 economically obtained, results are required. The subject
invention provides faster results due to the basic

simplicity of piezoelectric mass detection and can be
applied to mixed bacterial or viral cultures whereas non-
hybridization methods of identifying bacteria and viruses
generally require that a pure culture (non-mixed) be
5   obtained. In addition, the present invention should
provide an advantage over older methods of pathogen
identification. The older methods are based on the
observation of phenotypes which can fail to correctly
identify mutated species which can otherwise be detected
10   by hybridization techniques. Moreover, mutagenic dyes, as
used in fluorescence assay methods, and ^{32}P or equally
hazardous isotopes, as used in radio assay methods, are
avoided.

15   Other diagnostic uses are also contemplated as being
within the scope of the present invention. For example,
it is contemplated that the present invention will be
useful for diagnosing the presence of nucleic acid-
containing etiological agents, testing bacterium to
20   determine the presence of resistance to an antibiotic,
detecting genetic disorders, diagnosing tumor cells and
other similar techniques where polynucleotide
hybridization is employed.

25   The following examples are given to more fully
illustrate the present invention, and shall not be con-
strued as limiting the scope of the invention in any way.
These examples are representative of the immobilization of
polynucleotides on crystals, their subsequent hybridi-
30   zation and the detection of hybridization by their piezo-
electric resonance. Therefore, the method is not limited
to only those DNAs or RNAs enumerated.

EXAMPLES

The procedures used in the following Examples follow
the general procedures discussed above. The procedures
5   were carried out in parallel on two separate crystals, one
of which was designated as a control. The frequency
shifts obtained upon hybridization are expressed relative
to the control.

10   The control was processed in the same manner as the
test sample except that the conditions for the control
were adjusted so that significant hybridization would not
occur.

15   The results are expressed as follows:

$$\Delta Hz = (Hz_2 - Hz_3) \text{ control} - (Hz_2 - Hz_3) \text{ test}$$
sample where:

Hz_2 = the resonance frequency of the prepared
20   crystal, and
Hz_3 = the resonance frequency of the crystal
following the incubation period.

Hybridization is generally indicated when ΔH has a value
25   from minus a few hundred to minus several thousand Hertz.
The reproducibility of ΔHz depends on various factors such
as the reproducibility of the polynucleotide immobiliza-
tion and the subsequent hybridization conditions and
reactions.
30

RNA Hybridization Detected by Piezoelectric Resonance

It is well known that hybridization will occur
between polyinosinic acid (poly I) and polycytidylic acid
(poly C) to form a double-stranded synthetic polymer (poly
35   I:C). However, hybridization will not occur between
polyuridylic acid (poly U) and polyinosinic acid (poly I).

Examples 1-3 below demonstrate the ability to measure
specific hybridization which can occur on the crystal
surface.

5   Example 1

Polycytidylic acid was hybridized with Polyinosinic
acid attached to Poly(butyl methacrylate) substrate as
follows:

10

10 microliters of poly(butyl methacrylate) (0.1% in
ethyl acetate) was applied to both sides of the crystal
and dried. 10 microliters of triazido trinitro benzene
linker (0.1% in chloroform) was applied to both sides of

15   the crystal. 5 microliters of 1 milligram/milliliter poly
I in buffer A (0.14 Molar sodium phosphate, pH 6.8) was
applied to both sides of the polymer coated crystal and
irradiated at 300 nanometers for 30 minutes on each side.
5 microliters of 1 milligram/milliliter poly C in buffer A

20   was applied for hybridization. Hybridization was done at
46°C for 25 minutes under a glass dome.

The control crystal was carried through the identical
procedure without polyinosinic acid

25

FIRST RESULT: . ΔHz = -613 versus control
SECOND RESULT: ΔHz = -686 versus control

Example 2

30

Polycytidylic acid was hybridized with Polyinosinic
acid attached to a polyurethane substrate as follows:

5 microliters of polyurethane (0.2% in dimethyl-
35   formamide) was applied to both sides of the crystal and
dried. 5 microliters of 2,6-bis(4-azidobenzylidene)-

cyclohexanone linker (0.1% in acetone) was applied to both
sides of the crystal. 5 microliters of 5 milligram/milli-
liter poly I in buffer B (0.10 Molar sodium, 0.01 Molar
phosphate, pH 7.8) was applied to both sides of the
5   polymer coated crystal and irradiated at 330 nanometers
for 30 minutes on each side. 5 microliters of 2.0×10^{-3}
Molar poly C (0.65 milligram/milliliter) in buffer B was
applied to both sides of the crystal for hybridization.
The crystal was heated with poly C to 65°C for 30 minutes
10   and the hybridization was carried out at room temperature
for 2 hours with silicone rubber domes.

The control crystal was carried through an identical
procedure without polyinosinic acid.
15
RESULT: ΔHz = -1237 versus control

Example 3

20   Polycytidylic acid was hybridized with polyinosinic
acid attached to a polyurethane substrate with cyclohexene
reactive site mask as follows:

10 microliters of polyurethane (0.2% in
25   dimethylformamide) were added to both sides of the crystal
and dried. 10 microliters of 2,6-bis(4-azidobenzylidene)-
cyclohexanone linker (0.2% in acetone) was then added to
both sides of the crystal. 5 microliters of 2.0×10^{-3}
Molar poly I (0.70 milligram/milliliter) in buffer B (0.10
30   Molar sodium, 0.10 Molar phosphate, pH 7.8) was applied to
both sides of the polymer coated crystal and irradiated at
366 nanometers for 0.5 hours. 10 microliters of cyclo-
hexene was applied to each side of the crystal and ir-
radiated at 366 nanometers for 10 minutes on each side. 5
35   microliters of 2.0×10^{-3} Molar poly C (0.65 milli-
gram/milliliter) in buffer B was applied to both sides of

the crystal for hybridization. The crystal was heated to
60°C with the poly C for 30 minutes and then incubated for
2 hours at room temperature for hybridization.

5   The control crystal was carried through the identical
procedure, but using 5 microliters of 2.0×10^{-3} Molar
polyuridylic acid (0.65 milligram/milliliter) in place of
polycytidylic acid.

10   RESULT: ΔHz = -227 Hz versus control

         The control ($Hz_3 - Hz_2$) was +18 Hz demonstrating that
there was not apparent hybridization between the poly-
inosinic acid immobilized on the crystal and polyuridylic
15   acid present in solution. This is consistent with the
known specificity of polyinosinic acid for hybridization
to poly C but not to poly U.

DNA Hybridization Detected by Piezoelectric Resonance
20

Example 4

         Salmonella typhimurium (S. typhimurium) DNA was
hybridized to S. typhimurium DNA attached to a poly(butyl
25   methacrylate) substrate as follows:

         10 microliters of a 100:1 (Volume/Volume) solution of
poly(butyl methacrylate) (0.1% in ethyl acetate) and
dimethoxyphenylacetophenone (0.1% in acetone) was applied
30   to the crystal. 10 microliters of 1 milligram/milliliter
S. typhimurium DNA (denatured by boiling and then quick
cooling at 4°C) in buffer A (0.14 Molar sodium phosphate,
pH 6.8) was applied to one side of the crystal and ir-
radiated at 365 nanometers for 2 hours. The crystal was
35   rinsed with distilled water and dried. In the case of
RESULT 1 below, an additional 10 microliters of denatured

S. typhimurium DNA was applied and irradiated as above.
Hybridization was carried out with 10 microliters of
1 milligram/milliliter melted S. typhimurium DNA (heated
at 100°C and then cooled to 65°C). Incubation was at 65°C
5 for 2 hours with a silicone rubber dome.

The control crystal was carried through the identical
procedure but incubated with 20 microliters of 0.4 mil-
ligram/milliliter of melted calf thymus DNA instead of
10 melted S. typhimurium DNA during the hybridization step.

FIRST RESULT: ΔHz = -779 versus control
SECOND RESULT: ΔHz = -1013 versus control

15 Example 5

S. typhimurium DNA was hybridized to S. typhimurium
DNA attached to a poly(butyl methacrylate) substrate as
follows:
20

10 microliters of a 100:1 (Volume/Volume) solution of
poly(butyl methacrylate) (0.1% in ethyl acetate) and
dimethoxyphenylacetophenone (0.1% in acetone) was applied
to the crystal. The crystal was then dipped into the
25 poly(butyl methacrylate):dimethoxyphenylacetophenone
solution having the above composition. 10 microliters of
1 milligram/milliliter S. typhimurium (denatured by
boiling and then quick cooling at 4°C) in buffer A (0.14
Molar sodium phosphate, pH 6.8) was applied to one side of
30 the crystal and irradiated at 365 nanometers for 2 hours. .
Hybridization was carried out with 10 microliters of
1 milligram/milliliter melted S. typhimurium DNA (heated
at 100°C and then cooled to 65°C) and incubated for 2
hours with a silicone rubber dome.
35

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The control crystal was carried through the identical procedure, but denatured calf thymus DNA (25 microliters of 0.4 milligram/milliliter in buffer A) was used for immobilization instead of denatured S. typhimurium DNA.

5

RESULT: ΔHz = -833 versus control

Example 6

10   S. typhimurium DNA was hybridized to endonuclease restricted S. typhimurium DNA inserted into single-stranded M13 viral DNA attached to poly(butyl methacrylate) as follows:

15   5 microliters of a 100:1 (Volume/Volume) solution of poly(butyl methacrylate) (0.1% in ethyl acetate) and dimethoxyphenylacetophenone (0.1% in acetone) was applied to the crystal. 10 microliters of 1 milligram/milliliter single-stranded M13 mp8 with Salmonella DNA insert in
20 buffer B (0.10 Molar sodium, 0.10 Molar phosphate, pH 7.8) was applied to a polymer coated crystal and irradiated at 365 nanometers for 2 hours. 10 microliters of 1 milligram/milliliter blender sheared (4 minutes), melted S. typhimurium DNA was applied for hybridization at 65°C for
25 2 hours using silicone rubber domes.

Control 1:   · The crystal was carried through an identical procedure but using 10 microliters of 1 milligram/milliliter of blender sheared, melted calf thymus
30 DNA for the hybridization step instead of S. typhimurium DNA.

Control 2:   The crystal was carried through an identical procedure, but using buffer B instead of M13 mp8 DNA
35 with Salmonella DNA insert, and using 10 microliters of

1 milligram/milliliter blender sheared, melted calf thymus
DNA for the hybridization step instead of S. typhimurium
DNA.

5   RESULT: ΔHz = -557 versus control 1
             ΔHz = -875 versus control 2

CLAIMS:

1.   A component for use in detecting polynucleotide
hybridization comprising:

5

        (a)   a piezoelectric crystal; and

        (b)   a polynucleotide immobilized on a surface of
              said crystal.

10

2.   The component of claim 1 further comprising means for
determining the resonance frequency of said crystal.

15

3.   The component of claim 2 wherein said means for
determining the resonance frequency of said crystal
includes electrode material deposited on at least a
portion of opposite surfaces of said crystal.

20

4.   The component of claim 3 wherein a substantial
portion of said polynucleotide is immobilized on that
portion of said crystal surface comprising said electrode
25   material.

5.   The component of claim 1 or 4 wherein said crystal
surface to which said polynucleotide is immobilized
30   further comprises a polymeric substrate intermediate
between the crystal and polynucleotide layer.

6.   The component of claim 1 or 4 wherein said
polynucleotide is immobilized on a surface of said crystal
by incorporating a linker between said polynucleotide and
said surface to which said polynucleotide is to be im-
5 mobilized.

7.   The component of claim 1 or 4 wherein said
polynucleotide is immobilized on a surface of said crystal
10 by enzymatically ligating said polynucleotide using DNA or
RNA ligase to a different polynucleotide in order to then
covalently bond said different polynucleotide by chemical
reaction to a functional group which is in turn chemically
bonded to said crystal surface.
15

8.-   The component of claim 1 wherein said polynucleotide
is DNA or RNA.

-20.

9.   The component of claim 3 wherein said electrode
material comprises a metal selected from gold, silver,
aluminum, nickel, chromium, titanium, tantalum.

25

10.   The component of claim 5 wherein said polymeric
substrate is selected from a class of polymers
characterized by hydrolytically stable, hydrophobic
backbones substituted with reactive pendant groups.
30

11.   The component of claim 10 wherein said polymer is
selected from copolymers of styrene and p-x-methylstyrene,
ethylene or propylene and N-(6-x-hexyl)-acrylamide,
35 alkylated siloxanes and 6-x-hexyl and alkyl substituted

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siloxanes, where X is a reactive group capable of
combining with polynucleotides or derivatized
polynucleotides.

5

12. The component of claim 5 wherein said polymeric sub-
strate comprises a synthetic polynucleotide.

10 13. The component of claim 5 wherein said polymeric sub-
strate comprises a polymer selected from poly(butyl
methacrylate), polyurethane.

15 14. The component of claim 6 wherein said linker is an
organic compound characterized by the presence of an azido
group.

20 15. The component of claim 14 wherein said linker is
selected from 2,6-bis(4-azidobenzylidene)-cyclohexanone,
triazido trinitro benzene.

25 16. A device for detecting the relatedness between a
given polynucleotide and another polynucleotide, compris-
ing a piezoelectric crystal, a surface of said crystal
having said given polynucleotide attached thereto, said
crystal being in electrical cooperation with a means for
30 determining the resonance frequency of said crystal.

17. A device for detecting polynucleotide hybridization
comprising:

35

(a) a piezoelectric crystal;

(b) a polynucleotide attached to a surface of said
     crystal; and

5

(c) a means for determining the resonance frequency
     of said crystal.

10 18. The device of claim 17 wherein said surface to which
    a substantial portion of said polynucleotide is attached
    comprises an electrode material deposited on at least a
    portion of said crystal.

15

    19. The device of claim 17 wherein said surface to which
    a substantial portion of said polynucleotide is attached
    comprises a polymer coated on at least a portion of said
    crystal.
20

    20. The device of claim 17 wherein said crystal is a
    quartz crystal.

25

    21. The device of claim 18 wherein said electrode mater-
    ial is a metal selected from gold, silver, chromium,
    nickel, aluminum, titanium tantalum.

30

    22. The device of claim 17 wherein said polynucleotide is
    attached to said surface by a linker.

23.   The device of claim 19 wherein said polymer is
selected from a class of polymers characterized by
hydrolytically stable, hydrophobic backbones substituted
with reactive pendant groups.
5 .

24.   The device of claim 23 wherein said polymer is
selected from copolymers of styrene and p-x-methylstyrene,
ethylene or propylene and N-(6-x-hexyl)-acrylamide,
10   alkylated siloxanes and 6-x-hexyl and alkyl substituted
siloxanes, where X is a reactive group capable of
combining with polynucleotides or derivatized
polynucleotides.

15
25.   The device of claim 19 wherein said polymer is
selected from the group comprising poly(butyl
methacrylate), polyurethane.

20
26.   The device of claim 19 wherein said polymer is a
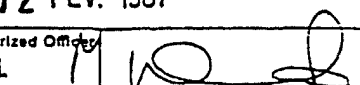synthetic polynucleotide.

25   27.   The device of claim 22 wherein said linker is
selected from the group comprising organic compounds
characterized by the presence of an azido group.

30   28.   A method of detecting polynucleotide hybridization
between a first polynucleotide and a second polynucleotide
comprising the steps of:

-25-

(a)   detecting a first resonance frequency of a
      piezoelectric crystal wherein a first poly-
      nucleotide is attached to a surface of said
      crystal;

5

(b)   exposing said attached first polynucleotide to a
      separate source of a second polynucleotide for a
      sufficient length of time and under conditions
      suitable for hybridization to occur between said
10   first polynucleotide and said second polynucleo-
      tide;

(c)   detecting a second resonance frequency of said
      crystal following said hybridization step; and

15

(d)   comparing said first and said second resonance
      frequencies.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 86/01861

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁴: C 12 Q 1/68, G 01 N 27/00//C 12 Q 1/70

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
IPC⁴	C 12 Q; G 01 N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category *	Citation of Document, ¹¹ with Indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	US, A, 4314821 (T. RICE) 9 February 1982, see the whole document	1-10,16-28
Y	WO, A, 80/02201 (MINNESOTA MINING & MANUFACTURING CO.) 16 October 1980, see the whole document	1-10,16-28
Y	WO, A, 80/02158 (MINNESOTA MINING & MANUFACTURING CO.) 16 October 1980, see the whole document	1-10,16-28
Y	US, A, 4242096 (R. OLIVERIA et al.) 30 December 1980, see the whole document	1-10,16-28
Y	Chemical Abstracts, volume 103, no. 19, 11 November 1985, Columbus, Ohio (US) S. Wang: "Genetic substances and material sciences", see page 277, abstract 155873f, & Yichuan, 1985, 7(2), 46-48	1-10-16-28

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16th January 1987	12 FEV. 1987

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	M. VAN MOL

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
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A	WO, A, 85/02627 (GENETICS INTERNATIONAL INC.) 20 juni 1985	

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON

INTERNATIONAL APPLICATION NO.   PCT/US 86/01861 (SA   14696)
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This Annex lists the patent family members relating to the
patent documents cited in the above-mentioned international
search report. The members are as contained in the European
Patent Office EDP file on 29/01/87

The European Patent Office is in no way liable for these
particulars which are merely given for the purpose of
information.

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		AU-B-	538366	09/08/84
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